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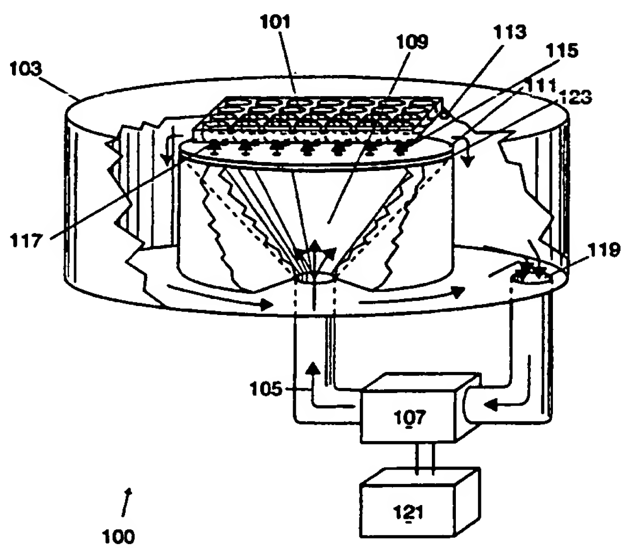

(57) Abstract

Apparatus is disclosed that thermally cycles samples between at least two temperatures. The apparatus operates by impinging fluid jets onto the outer walls of a sample-containing region. Because the impinging fluid jets provide a high heat transfer coefficient between the jet and the sample-containing region, the sample-containing regions are uniformly cycled between the two temperatures. The heat exchange rate between the jets and the sample regions is substantially greater than in the case of laminar flow.

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APPARATUS FOR A FLUID IMPINGEMENT THERMAL CYCLER

Field of the Invention

This invention relates to a method and apparatus that facilitates the rapid, uniform temperature cycling of samples. More particularly, the invention is directed to an apparatus for performing DNA amplification.

Background

There are a variety of investigative settings in which many oligonucleotide or polynucleotide samples, or specific DNA fragments within a sample mixture, are amplified by polymerase chain reaction (PCR). For example, DNA samples contained in the wells of a microtiter plate can be PCR-amplified as an array. In still another setting, it may be desirable to compare the amplification products of one or more DNA fragments contained in different tubes in a tube holder.

If the amplified fragments from the different samples are to be compared, either for fragment size or quantity, it is desirable to conduct the PCR amplification of each sample under substantially identical conditions. This means that the concentration of PCR reagents, as well as the thermal cycling times and temperatures, should be carefully controlled and uniform among all of the samples.

Heretofore, a variety of devices have been used or proposed for carrying out PCR reactions simultaneously in a plurality of structures. Typically, these devices involve a heat block placed against the wells of a microtiter plate, or a heat block designed to hold a plurality of sample tubes. The block, in turn, is alternately heated and cooled by circulating a heating fluid through the block, or by heat conduction to the block. It is difficult to achieve uniform heating and cooling cycles in this type of device, due to uneven heat transfer rate and temperatures within the block and due to the difficulty of providing a good thermal connection between the block and the wells or tubes.

It has also been proposed to circulate a temperature-controlled fluid (such as air or water) past sample tubes as shown by US Patent number 5,187,084 to Hallsby. This allows a higher frequency for temperature cycling as the temperature of the flowing fluid is easier to control than that of the block. However, this approach results in temperature gradients on the sample tubes because the fluid flow around a tube causes the temperature of the fluid flowing next to the sample tube to be affected by the temperature of the sample tube itself.

Thus, the fluid flow adjacent to the tube at the upstream part of the tube is at a different temperature than the fluid adjacent to the tube at the downstream portion of the tube. In addition, temperature gradients occur within the sample tube because the heat transfer where the fluid impinges the tubes is different from the heat transfer where the fluid flows past the tubes.

Summary of the Invention

The invention includes an apparatus for thermally cycling a plurality of samples between at least two temperatures. Each of the samples is held in one of a plurality of sample regions in an array. Each of the sample regions in the array defines an outer heat-exchange wall expanse. The apparatus includes a source that provides a pressurized fluid at selected first and second temperatures. The apparatus also includes a chamber that contains a structure adapted to support the array. The chamber contains a manifold that receives the pressurized fluid and distributes the same as a plurality of fluid jets directed against, and substantially normal to, the sample wall expanses, when the array is held by the structure. The pressurized fluid impinging on the wall expanses creates substantially uniform heat exchange between the fluid jets and the samples. The apparatus also includes an outlet for venting the fluid from the fluid jets out of the chamber.

In one aspect, the apparatus includes the array of sample regions, such as a microtiter plate having a plurality of sample wells, or a plurality of tubes held in a tube holder. In an alternative aspect, the apparatus is adapted for use with the array.

The foregoing and many other aspects of the present invention will become more fully apparent when the following detailed description of the preferred embodiments is read in conjunction with the various figures.

Description of the Drawings

Fig. 1 illustrates an apparatus for thermal cycling an array of samples in accordance with an embodiment of the invention; Fig. 2 is an enlarged fragmentary portion of an impingement plate, associated structures and a microtiter plate as used in the Fig. 1 apparatus; Fig. 3 illustrates an apparatus for thermal cycling an array of samples within tubes in accordance with an embodiment of the invention; and Fig. 4 is an enlarged fragmentary portion of an shaped impingement plate, associated structures and tube array as used in the Fig. 3 apparatus.

Description of the Preferred Embodiments

The invention is a high performance thermal cycling device used to uniformly change the temperature of an array of samples. One use for the invention is that of PCR amplification.

Fig. 1 illustrates a thermal cycling apparatus, indicated by general reference character **100**, for thermally cycling samples between at least two temperatures. Thermal cycling apparatus **100** is designed to be used with an array **101** that has a plurality of sample regions. Array **101** is subsequently described with respect to Fig. 2. Thermal cycling apparatus **100** includes a closed loop fluid chamber **103** that circulates a pressurized fluid **105**. Closed loop fluid chamber **103** is sealed so as to contain the fluid. Pressurized fluid **105** is pressurized by a source **107**. Source **107** also adjusts the temperature of pressurized fluid **105**. Pressurized fluid **105** enters a manifold **109** that includes an impingement plate **111**. In manifold **109** pressurized fluid **105** is uniformly distributed to impingement plate **111**. Array **101** is supported in closed loop fluid chamber **103** by a structure **113**.

Pressurized fluid **105** flows through holes in impingement plate **111** creating fluid jets **115** (indicated by the arrows extending upward from impingement plate **111**) that impinge on an outer heat-exchange wall expanse **117**. After fluid jets **115** impinge on outer heat-exchange wall expanse **117** the spent fluid that formed the jets flows to, and through, an outlet **119** to complete the fluid loop to source **107**. At source **107**, the spent fluid is again pressurized and heated or cooled. Source **107** is controlled by a control unit **121**, using methods well understood in the art, and adjusts the temperature and pressure of pressurized fluid **105**.

Source **107** contains an impeller (not shown) for pressurizing the spent fluid. It also contains a mechanism (not shown) for heating and cooling the spent fluid. The impeller is positioned after the heating/cooling mechanism so that it thoroughly mixes the temperature controlled spent fluid. Thus, pressurized fluid **105** does not have thermal gradients. To further minimize temperature gradients in pressurized fluid **105** the walls of manifold **109** can be insulated.

In some embodiments, impingement plate **111** can be removed from the rest of manifold **109** and replaced by a differently shaped plate. In other embodiments impingement plate **111** is formed by manifold **109**. Further, some embodiments may have a

sterile filter **123** within manifold **109** prior to impingement plate **111** to filter pressurized fluid **105**.

Fig. 2 illustrates an enlarged portion of array **101** of Fig. 1 as indicated by general reference character **124**. In this figure, array **101** is a microtiter plate. Microtiter plate **101** has a plurality of wells **125** (the sample regions) for holding a plurality of samples **127** respectively. Each of plurality of wells **125** has a well bottom surface **129**. In this embodiment, well bottom surface **129** of each of plurality of wells **125** make up outer heat-exchange wall expanse **117** of thermal cycling apparatus **100** of Fig. 1. Apertures in flat impingement plate **111** generate fluid jets **115**. Each of fluid jets **115** impinges on well bottom surface **129** associated with that particular fluid jet. Thus, well bottom surfacc **129** is tightly coupled (thermally) to the temperature of its respective fluid jet(s).

Spent fluid **131**, from fluid jets **115** that has impinged on outer heat-exchange wall expanse **117**, flows in a laminar manner past other of plurality of wells **125** to outlet **119**. Because the heat transfer between a laminar flow fluid and a surface is several times less than that between a directly impinging fluid and a surface, the temperature of the spent fluid does not affect the temperature of other of plurality of wells **125**. The heat transfer from of the impinging fluid jet to the surface also is significantly greater than the heat transfer between the surface and spent fluid **131** even when spent fluid **131** flows past the surface in a fully developed turbulent flow. Thus, each of plurality of wells **125** has the same temperature and there is no significant temperature gradient between any two of plurality of wells **125**.

Closed loop fluid chamber **103** is closed by microtiter plate **101** so that the top of microtiter plate **101** is not exposed to the fluid. Microtiter plate **101** is held on closed loop fluid chamber **103** by structure **113** that includes a fluid-tight plate seal **133**. Fluid-tight plate seal **133** seals the interface between microtiter plate **101** and closed loop fluid chamber **103** so that the fluid does not escape the chamber. Because microtiter plate **101** is not completely immersed in the fluid, the tops of plurality of wells **125** may be left open or closed with an inexpensive cap. One skilled in the art will understand that if plurality of wells **125** are sealed against the fluid that microtiter plate **101** can be immersed within the fluid.

Each of fluid jets **115** is formed by passing pressurized fluid **105** through an aperture such as an orifice, shaped nozzle, or formed slot in impingement plate **111**. Impingement

plate **111** is separated from outer heat-exchange wall expanse **117** by a distance that is on the order of two to ten times the width of fluid jets **115** dependent on the fluid use and the desired pressure drop. The pressure of pressurized fluid **105** is such that fluid jets **115** formed by the apertures reach well bottom surface **129** and form fully turbulent flow at well bottom surface **129**. The heat transfer efficiency of impinging fluid jets **115** on well bottom surface **129** is a function of the power applied to the impeller. The shape of the apertures that form fluid jets **115** need not be round. One skilled in the art will understand that more than one of the fluid jets **115** may be directed to a particular well bottom surface **129**. Conversely, only one of the fluid jets **115** may be directed to impinge on multiple well bottom surfaces so long as the temperature gradients between the well bottom remain within tolerance.

In addition, one skilled in the art will understand that impingement plate **111** can be constructed to be removed from manifold **109** or formed as part of manifold **109**. In the embodiment shown in Fig. 2 impingement plate **111** is removable from manifold **109** and the resulting interface is sealed by a fluid-tight manifold seal **135**.

Fig. 3 illustrates a thermal cycling apparatus, indicated by general reference character **300**, for thermally cycling samples held in tubes between at least two temperatures. Thermal cycling apparatus **300** is designed to be used with a tube array **301** that uses a plurality of tubes as the sample regions. Tube array **301** is subsequently described with respect to Fig. 4. Thermal cycling apparatus **300** includes a closed loop fluid chamber **303** that circulates a pressurized fluid **305**. Closed loop fluid chamber **303** is sealed so as to contain the fluid. Pressurized fluid **305** is pressurized by a source **307**. Source **307** also adjusts the temperature of pressurized fluid **305**. Pressurized fluid **305** enters a manifold **309** that incorporates a shaped impingement plate **311**. Tube array **301** is supported in closed loop fluid chamber **303** by a structure **313** such that each of the tubes in tube array **301** extend into a pocket (shown in, and subsequently described with respect to Fig. 4) formed by shaped impingement plate **311**. Pressurized fluid **305** is uniformly distributed to shaped impingement plate **311** by manifold **309**. Pressurized fluid **305** flows through holes in shaped impingement plate **311** creating fluid jets (shown in, and subsequently described with respect to Fig. 4). The spent fluid that formed the fluid jets flows to, and through, an outlet **315** to complete the fluid loop to source **307**. At source **307**, the spent fluid is again pressurized and heated or cooled. Source **307** is controlled by a control unit **317**, using

methods well understood in the art, and adjusts the temperature and pressure of pressurized fluid **305**.

Some embodiments may have a sterile filter **319** within manifold **309** prior to shaped impingement plate **311** to filter pressurized fluid **305**.

Source **307** contains an impeller (not shown) for pressurizing the spent fluid. It also contains a mechanism (not shown) for heating and cooling the spent fluid. The impeller thoroughly mixes the spent fluid so that pressurized fluid **305** does not have thermal gradients. The impeller is positioned after the heating/cooling mechanism so that it thoroughly mixes the temperature controlled spent fluid. Thus, pressurized fluid **305** does not have thermal gradients. To further minimize temperature gradients in pressurized fluid **305** the walls of manifold **309** can be insulated.

Fig. 4 illustrates an enlarged portion of tube array **301** of Fig. 3 as indicated by general reference character **320**, that includes a support plate **321** that rigidly holds a plurality of tubes **323** in tube array **301**. In the embodiment shown, each of plurality of tubes **323** is molded in support plate **321**. One skilled in the art will understand that other techniques exist to rigidly attach each of plurality of tubes **323** to support plate **321** such as by the use of a threaded connection.

Each of plurality of tubes **323** has an elongated sample-holding portion **327** that extends into one of a plurality of pockets **329** formed by shaped impingement plate **311**. Each of plurality of pockets **329** has one or more apertures **331** each of which form a fluid jet **333**, from pressurized fluid **305**) that impinges on elongated sample-holding portion **327** of one of plurality of tubes **323** at approximately ninety degrees from the surface of elongated sample-holding portion **327**. The outside of elongated sample-holding portion **327** is the outer heat exchange wall expanse. Impinging fluid jet **333** on the outer heat-exchange wall expanse efficiently transfers heat between fluid jet **333** and the outer heat-exchange wall expanse. Each of plurality of tubes **323** holds a sample **335** that is cycled between at least two temperatures dependent on the temperature of fluid jet **333**. Spent fluid **337** from fluid jet **333** flows out of each of plurality of pockets **329** and past the non-sample-holding portion of plurality of tubes **323** in a laminar-flow manner. Because the heat transfer coefficients of a laminar flow is so much less than that of an impinging flow, the spent fluid does not affect the temperature of the samples held in the other tubes. The heat transfer from of the impinging fluid jet to the surface also is significantly greater than the heat transfer between

the surface and spent fluid **337** even when spent fluid **337** flows past the surface in a fully developed turbulent flow. The fluid jets have a jet dimension. The diameter of the fluid jets range from 0.5 mm to approximately 2 mm depending on the fluid used and the pressure drop desired. Elongated sample-holding portion **327** is separated from the walls of one of plurality of pockets **329** by a distance on the order of two to ten times the jet diameter.

It will be appreciated from the foregoing that tube array **301** can be fully immersed within the fluid if plurality of tubes **323** are securely closed. In addition, one skilled in the art will understand that shaped impingement plate **311** can be constructed to be removed from manifold **309** or formed as part of manifold **309**. In the embodiment shown in Fig. 3, shaped impingement plate **311** is formed as part of manifold **309**. One skilled in the art will understand that some embodiments allow the different impingement plates to be interchangeable on the manifold. This allows the apparatus to be adapted to array configurations other than the ones describe herein.

It will be appreciated from the forgoing that the apparatus can be provided without the sample array and that the apparatus can be used with existing tubes, microtiter plates, or other similar sample-holding mechanisms. Because the heat transfer is a result of fluid jets impinging a surface, one skilled in the art will also understand that there is no need to attempt to form a high quality thermal seal between a thermal block and a sample container. Thus, the wall expanse can be irregular and does not rely on a mechanical contact thermal conduction path. It will also be appreciated that the invention contemplates many impingement jet configuration other than those described above. In particular, but without limitation, the invention contemplates applying impinging jets on both sides of a microtiter plate, to the lid of closed sample containers and to wells micro-machined in silicon or stamped in plastic.

The fluids most commonly used within the invention will be a gas, such as air, and a high heat capacity liquid, such as water. Liquid is the preferred fluid when using smaller geometry arrays or with rapid temperature ramp rates. In addition, a non-compressible liquid may be preferred if the temperature of the fluid jet is critical as a compressible gas cool as it expands and the temperature control mechanism does not take this cooling into account.

From the foregoing, it will be appreciated that the invention has the following advantages:

1. Direct heat exchange between the fluid and each sample region so as to

eliminate temperature gradients between the sample regions.

2. A fluid jet impinging at substantially ninety degrees to the heat exchange surface provides a more rapid and efficient heat transfer between the surface and the fluid than does laminar fluid flow adjacent to the heat exchange surface. Because the spent fluid from the impinging jets flows past other sample regions in such a laminar flow, the other sample regions are not affected by the temperature of the spent fluid. Thus, reducing temperature gradients between the sample regions in the array.

3. Allows precise controlled, uniform thermal cycling among separate sample regions such as a plurality of wells or tubes. This allows PCR amplification of separate samples under substantially identical conditions.

Although the present invention has been described in terms of the presently preferred embodiments, one skilled in the art will understand that various modifications and alterations may be made without departing from the scope of the invention. Accordingly, the scope of the invention is not to be limited to the particular invention embodiments discussed herein, but should be defined only by the appended claims and equivalents thereof.

Claims

What is claimed is:

1. An apparatus for thermally cycling a plurality of samples between at least two temperatures where each of said samples is held in one of a plurality of sample regions in an array, where each of said sample regions defines an outer heat-exchange wall expanse, said apparatus comprising:

a source for providing a pressurized fluid at a selected first and second temperatures; and a chamber containing:

(a) a structure adapted for supporting said array;

(b) a manifold for receiving said pressurized fluid and distributing same in the form of a plurality of fluid jets directed against said wall expanses, and substantially normal thereto, when said array is held by said structure, to produce substantially uniform heat exchange between said fluid jets and said samples; and

(c) an outlet for venting said fluid from said fluid jets out of said chamber.

2. The apparatus of claim 1 for use with a microtiter plate, comprising said array, wherein said sample regions are composed of a plurality of wells each having a bottom surface defining said wall expanse wherein said fluid jets impinge on said bottom surface when said plate is held by said structure.

3. The apparatus of claim 1 for use with a plurality of tubes, comprising said array, each of said tubes having an elongated sample-holding portion defining said wall expanse and said manifold having a plurality of pockets each adapted to enclose said wall expanse of one of said tubes;

each of said pockets distributing said fluid jets onto said wall expanse, said pockets open at the top to allow said fluid from said fluid jets to exit said pockets; and

said structure comprising a plate adapted to support said tubes descending into said pockets.

4. The apparatus of claim 1, wherein said samples are polynucleotides and said

apparatus is used for thermal cycling a polymerase chain reaction.

5. The apparatus of claim 1 further comprising fluid recycling means operatively connecting said outlet to said source for recycling said fluid there between.

6. The apparatus of claim 5 further comprising a sterile filter to filter said fluid.

7. The apparatus of claim 1, wherein said structure is adapted to form a fluid-tight seal between the structure and the array.

8. The apparatus of claim 1, wherein said pressurized fluid is a liquid.

9. The apparatus of claim 1, wherein said pressurized fluid is a gas.

10. An apparatus for thermally cycling a plurality of samples between at least two temperatures comprising:

a source for providing a pressurized fluid at a selected first and second temperatures; and a chamber containing:

(a) an array of a plurality of sample regions where each of said samples is adapted to be held in one of said sample regions and each of said sample regions defines an outer heat-exchange wall expanse;

(b) a structure supporting said array;

(c) a manifold for receiving said pressurized fluid and distributing same in the form of a plurality of fluid jets directed against said wall expanses and substantially normal thereto, to produce substantially uniform heat exchange between said fluid jets and said samples; and

(d) an outlet for venting said fluid from said fluid jets out of said chamber.

11. The apparatus of claim 10 wherein said array is contained in a microtiter plate wherein said sample regions are composed of a plurality of wells, each having a bottom surface defining said wall expanse wherein said fluid jets impinge on said bottom surface.

12. The apparatus of claim 10 wherein said array is formed by a plurality of tubes, each of said tubes having an elongated sample-holding portion defining said wall expanse and said manifold having a plurality of pockets each adapted to enclose said wall expanse of one of said tubes;

each of said pockets distributing said fluid jets onto said wall expanse, said pockets open at the top to allow said fluid from said fluid jets to exit said pockets; and

said structure comprising a plate to support said tubes descending into said pockets.

13. The apparatus of claim 10 wherein said samples are polynucleotides and said apparatus is used for thermal cycling a polymerase chain reaction.

14. The apparatus of claim 10 further comprising fluid recycling means operatively connecting said outlet to said source for recycling said fluid there between.

15. The apparatus of claim 14 further comprising a sterile filter to filter said fluid.

16. The apparatus of claim 10, wherein said structure is adapted to form a fluid-tight seal between the structure and the array.

17. The apparatus of claim 10, wherein said pressurized fluid is a liquid.

18. The apparatus of claim 10, wherein said pressurized fluid is a gas.

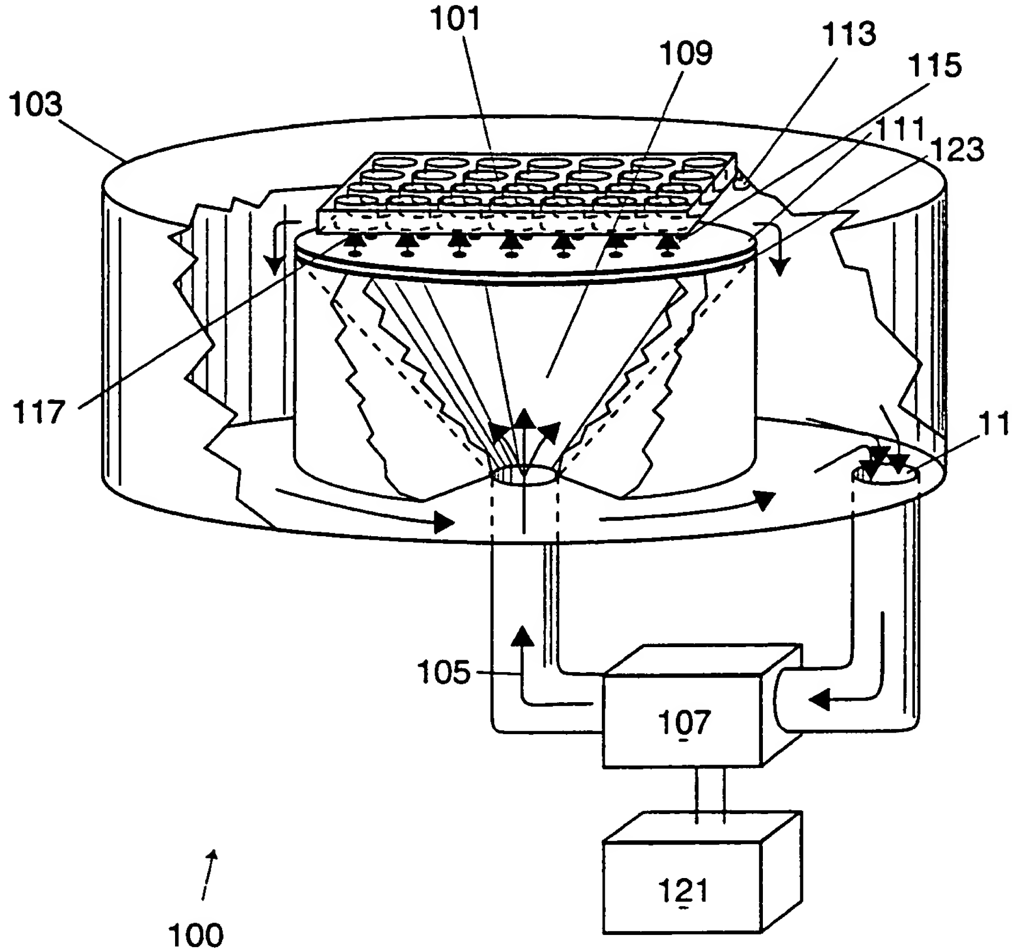

Fig. 1

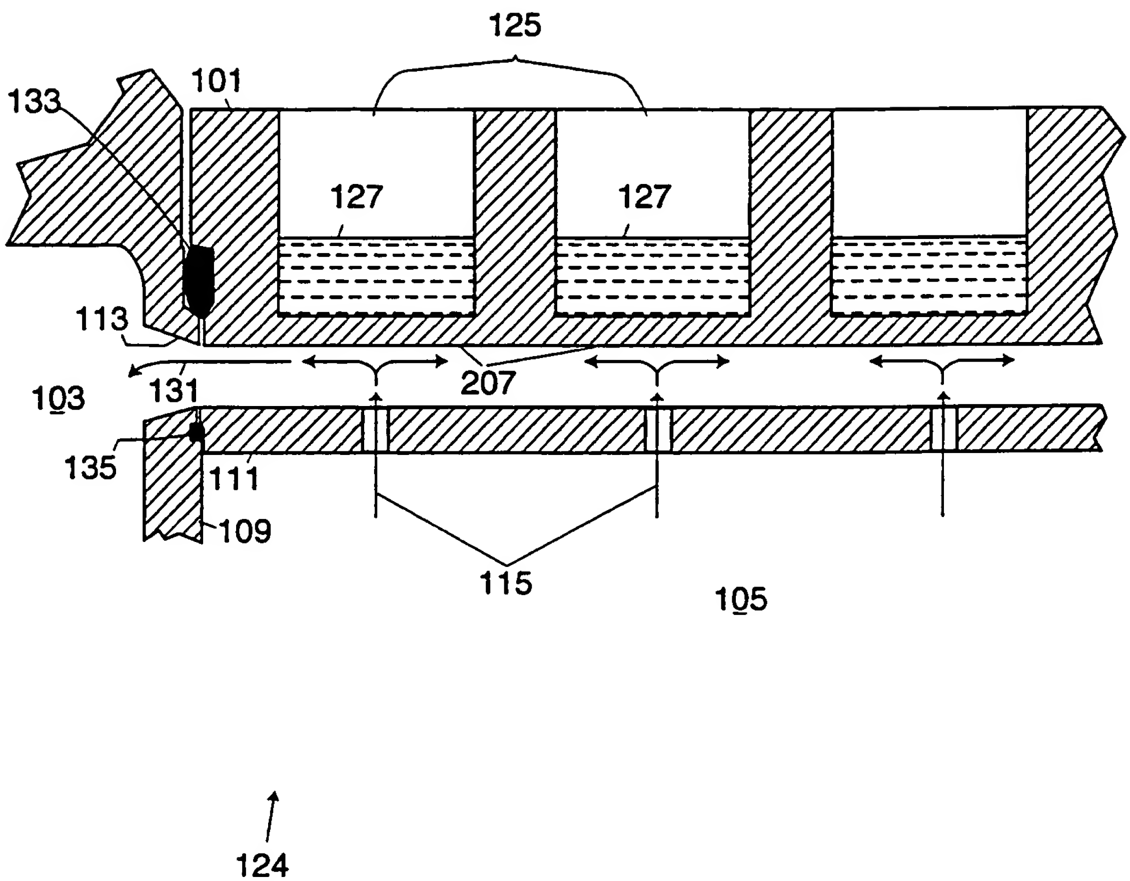

Fig. 2

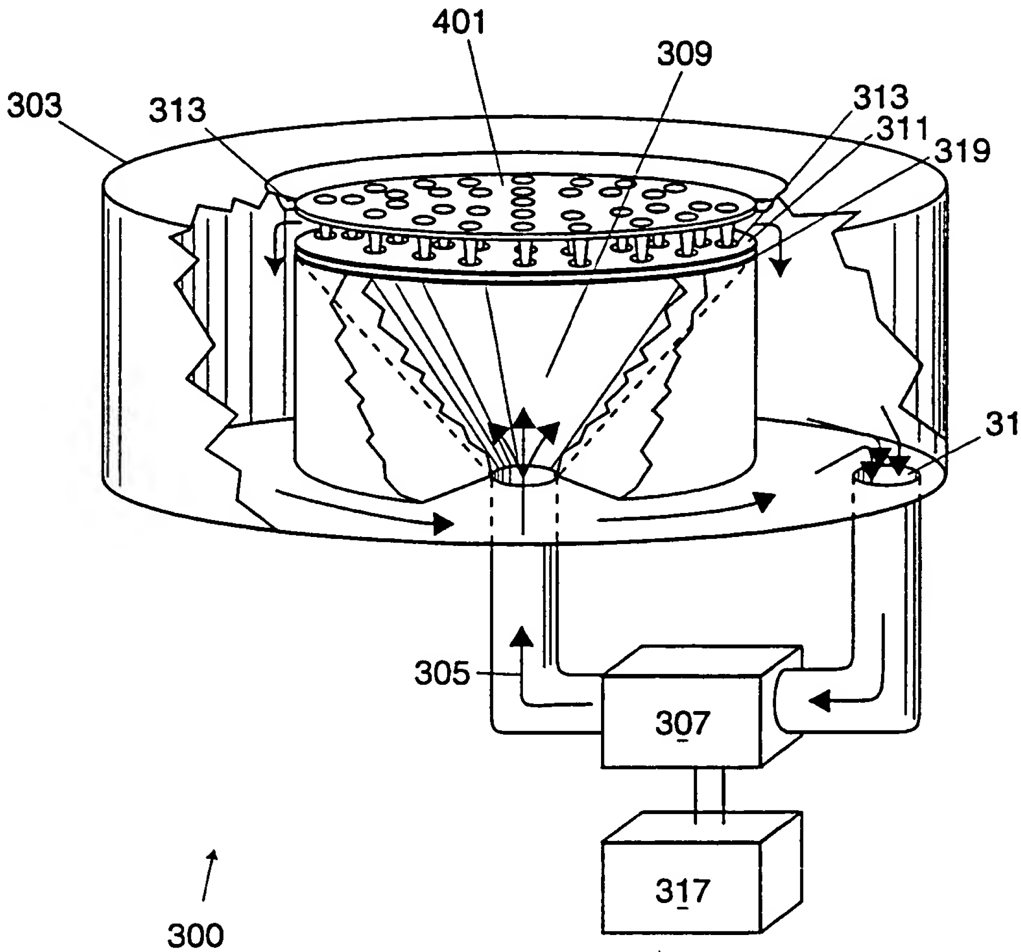

Fig. 3

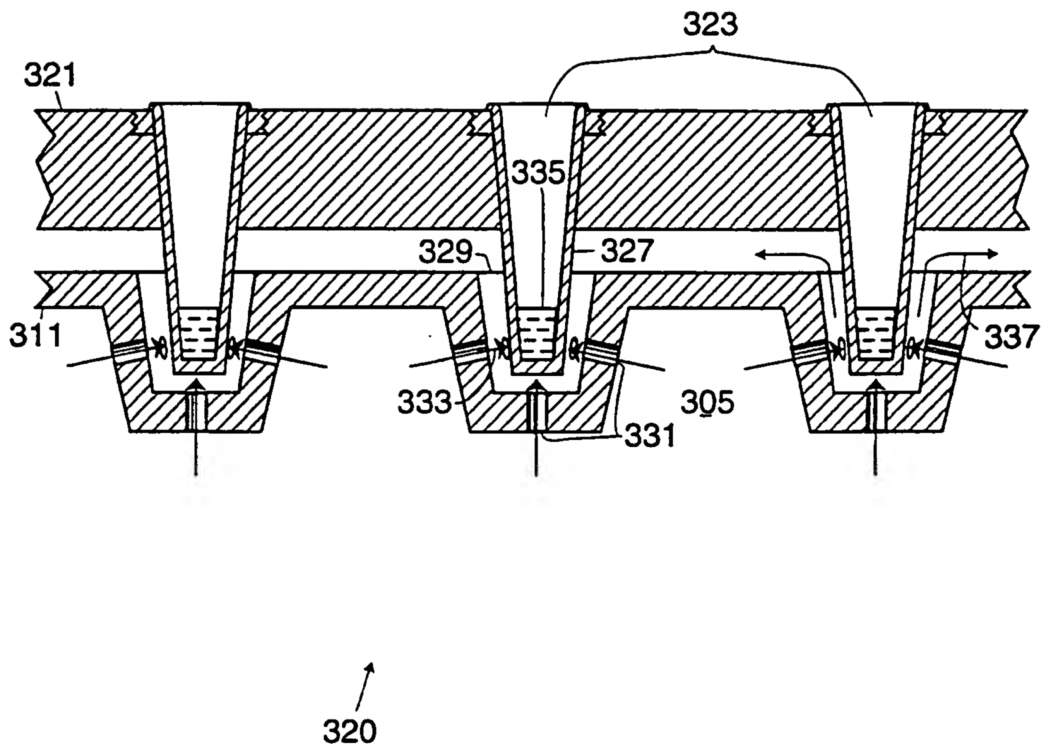

Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/20416

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L7/00 //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 09728 A (CENTRAL RESEARCH LAB LTD; CORLESS ANTHONY ROBERT (GB)) 12 March 1998 see page 15, line 10 - line 26; figures 3,4,6	1,2,4,5, 7,8,10, 11,13, 14,16,17
A	US 5 187 084 A (HALLSBY G ANDERS) 16 February 1993 cited in the application see column 4, line 5 - column 5, line 7; figure 1	1,3,4,9, 10,12, 13,18
A	EP 0 402 995 A (EASTMAN KODAK CO) 19 December 1990 see column 5, line 34 - line 53; figure 4 see column 7, line 27 - line 41; figure 6	1,4,9, 10,13,18

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 January 1999	19/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hodson, M

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP 0 281 958 A (HOECHST AG) 14 September 1988 see column 1, line 34 - line 41; figures	1,2, 9-11,18
A	DE 38 08 942 A (BIO MED GMBH GES FUER BIOTECHN) 28 September 1989 see column 4, line 49 - column 6, line 54; figure	1,3-5,8, 10, 12-14,17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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			FI	880988	A	08-09-1988
			JP	63242335	A	07-10-1988
DE 3808942	A	28-09-1989	NONE			